CHAPTER 4
RESULTS AND DISCUSSION

4.1 Nitrification/denitrification in airlift bioreactor systems

4.1.1 Packed bed external loop airlift bioreactor: Nitrogen compound removal

In this experiment, nitrification and denitrification of aquacultural synthetic seawater were performed in a novel designed packed bed external loop airlift bioreactor (PBABR). The packing employed in this system was a simple round-shape plastic bioball with a diameter of 2.5 cm and each packing had a surface area of 32.7 cm^2. The packing was designed for the immobilization of nitrifying and denitrifying bacteria. One primary advantage of the airlift system is that it provides both aerated and unaerated compartments, which can be served as nitrification and denitrification compartments in the same unit. The schematic diagram of this PBABR is illustrated in Figure 3.2. The recirculation of water was driven from the aeration in the riser where water was moved up the column and down through the downcomer section. In riser section, the high dissolved oxygen in wastewater was consumed by nitrifying bacteria, where ammonia was oxidized to nitrate. The low dissolved oxygen effluent flowed through downcomer section and ready for nitrate removal by denitrifiers.

The performance of this PBABR in removing total inorganic nitrogen in the synthetic wastewater is shown in Figure 4.1.1. The operation of the system was arranged into several time periods depending on the operating conditions. These conditions were altered to accomplish total inorganic nitrogen removal so that the wastewater can be reused back upstream. Details of these operating conditions are given in Table 4.1. Nitrification/denitrification rates during each time period are reported in Tables 4.2-4.3. Specific descriptions of the effect of parameters on these removal rates are given later in Sections 4.2 and 4.3.

This paragraph explains how to read Figures 4.1.1-4.1.4. The x-axis shows the time of experiment in PBABR and the y-axis shows the inorganic nitrogen

concentration (total inorganic nitrogen, ammonium-nitrogen, nitrite-nitrogen and nitrate-nitrogen in Figures 4.1.1 to 4.1.4, respectively). The concentration of inorganic nitrogen was measured at 3 sampling positions in each PBABR system including one in the left column (L), one in the central column (C), and the last one in the right column (R). The dash lines in these figures show the time that ammonium-nitrogen was added into the system. The description of "Replace entire solution" means the discharge of seawater and clean-up of bioballs in the PBABR system. The periodic clean-up of bioballs are necessary as excessive biofilm could suppress the activity of nitrifying bacteria. The alphabet A means the addition of nitrate-nitrogen and the position of 'A' in the figure is exactly at the time where nitrate-nitrogen was added. The addition of nitrate-nitrogen had the variation of 5 (A1), 10 (A2) and 20 (A3) mgNO_3-N/L.The alphabet B means the addition of methanol with a variation of volume ratio in system (as indicated in the label in the figure). Also the position of 'B' corresponds to the time where methanol was added into the system. The variation of methanol adding depended on the performance of PBABR and this is described later in this chapter.

Figure 4.1.1 shows an interesting result where the total inorganic nitrogen compounds content including (ammonium-nitrogen, nitrite-nitrogen and nitrate-nitrogen) during experiment period was totally removed by the PBABR. Every time the total nitrogen was removed, ammonia (and nitrate) was promptly added into the synthetic wastewater which rendered a high level of nitrogen content in the system (about 8-9 mg NH_4-N/L). There were times that total inorganic nitrogen could not be totally removed which was due to some improper conditions. This will be discussed in detail later on in the discussion. Note that the total nitrogen removal rate was calculated from

$$TNR = \frac{Ni\text{-}Nf}{t_f - t_i} \qquad (4.1)$$

where

TNR = Total nitrogen removal rate (mg N/L-h)

N_i = initial concentration of total nitrogen compounds (mg N)

N_f = final concentration of total nitrogen compounds (mg N)

t_i = initial time (h)

t_f = final time (h)

Experiment indicated that if the right conditions were selected, the total nitrogen contents were removed with an average rate of 0.33 mg N/L-h (maximum rate = 0.73 mg N/L-h and minimum rate = 0.09 mg N/L-h). It was then concluded that the system could be operated satisfactorily regarding the removal of inorganic nitrogen compounds.

Figure 4.1.2 shows the time profile of ammonium-nitrogen concentration. It was found that ammonium-nitrogen concentration was always brought down to zero meaning a 100% removal. This ammonium-nitrogen was believed to be consumed by nitrifying bacteria via nitrification reaction. Table 4.2 shows the ammonium-nitrogen removal rate in this airlift bioreactor over the various conditions in experimental period which was found to be in a range between 0.03-0.40 mg NH_4-N/L-h. The ammonium-nitrogen removal rate reported in this section was calculated from

$$NR = \frac{NH_4\text{-}N_i - NH_4\text{-}N_f}{t_f - t_i} \qquad (4.2)$$

where

NR = Total nitrogen removal rate (mg NH_4-N /L-h)

$NH_4\text{-}N_i$ = initial concentration of ammonium nitrogen (mg NH_4-N)

$NH_4\text{-}N_f$ = final concentration of ammonium nitrogen (mg NH_4-N)

t_i = initial time (h)

t_f = final time (h)

Figures 4.1.3 and 4.1.4 reveals the nitrite-nitrogen and nitrate-nitrogen concentrations obtained from this experimental. Most of the time these two nitrogen compounds were being totally removed by the PBABR. However, the rates at which nitrite and nitrate removal took place could not be determined from these experimental data because these two components were intermediates in nitrification/denitrification reactions and it was not possible to measure the exact time profile of these two components. It should be mentioned, nevertheless, that nitrite-nitrogen concentration level always remained below 2.0 mgNO_2-N/L where

nitrate-nitrogen varied significantly according to the operation conditions. Detail discussion will follow.

Overall, the performance of the PBABR in the removal of inorganic nitrogen compounds in synthetic seawater containing ammonia (representing the waste from aquaculture industry) was satisfactory. The system could be easily adjusted to give suitable conditions for both nitrification and denitrification to take place at 100% total nitrogen removal efficiency.

4.1.2 Comparative evaluation of nitrification/ denitrification performance

A comparison of nitrification rates of this work with those reported in literature reveals that maximum ammonia removal rate was in a moderate rate (see summary below). To generalize the results from this work, the nitrification rate was recalculated in terms of g NH_3-N per area of packing per day. In fact, our ammonia removal rate varied considerably. The low end occurred due to the clogging of biofilm in the packing as will be discussed later whereas the high end was usually obtained when the system was operated with high dissolved oxygen. The average ammonia removal rate from the PBABR was about 0.2-0.4 gNH_4-N/m^2-d which was among the high values reported in literature. The summary of the nitrification rate (NR) below illustrates that the nitrification rate takes place best in the trickling filter where the bacteria is exposed directly to the air. This leads to a high oxygen concentration which enhances the nitrification rate. However, this trickling filter system requires an extremely large space and involves complicated configuration.

Source	NR (gNH_4-N/m^2-d)	Reference
Tricking filter	0.149	Jaap and van Rijn (1995)
	0.1-0.2	Lakang and Kleppe (2000)
	0.22	Nihof (1995)
	0.15-0.43	van Rijn and Rivera (1990)
	0.4-1.4	Knosche (1994)
	0.24-0.55	Kamstra (1998)

Source	NR (gNH_4-N/m^2-d)	Reference
	0.28-0.69	Nihof and Bovendeur (1990)
	0.7-0.8	Nihof (1995)
	0.6-0.73	Bovendeur et al (1990)
	0.75	Otte and Rosentahl(1985)
	0.94-3.92	**Greiner and Timmons (1998)**
Submerge biofilter	0.056	Reyes and Lawson (1995)
	0.59	David et al (1998)
	0.083	MacMillan (1994)
	0.23	Tseng (1998)
	0.43	Wickin (1985)
	0.13-0.57	Greiner and Timmons (1998)
	0.69	Yang (1989)
	1.5	Shanableh (1998)
	0.58-1.35	Tschui (1994)
Bead filter (propeller washed)	0.28-0.55	Malone (1993)
	0.33-0.45	Sastry et al (1999)
Rotating biofilter contactor	0.257	Reyes and Lawson (1995)
Biodrum	0.4-1.6	Wortman and Wheaton (1991)
Sequencing batch reactor	1.86	Zhu and Chen (1999)
	0.15	Sliekers (2002)
Fluidized bed biofilter	0.21-0.27	Skolstrup et al (1998)
Activated sludge	0.82	Kim (2000)
Sequency batch reactor	0.15	Slieker (2002)
Moving bed bioreactor	0.59-0.75	Tale et al.(2003)
Airlift bioreactor	**0.06-0.87**	**This work**

For denitrification, as stated earlier that the denitrification rate (DNR) in the PBABR was rather difficult to determine, however, there were times that only nitrate existed in the system (during 150-190 hours and 1471-1544 hours). The rate at which nitrate decreased during these periods was, hence, employed as the denitrification rate. The summary of the DNR below indicates that the nitrate removal potential of the PBABR could compete well with the published

denitrification rates where the DNR from the PBABR was in the range obtained from the standard fixed film column. It should be mentioned here that the fluidized bed column provided an extremely high nitrate removal rate. A better mass transfer rate in the fluidized bed was believed to be the reason for this high rate.

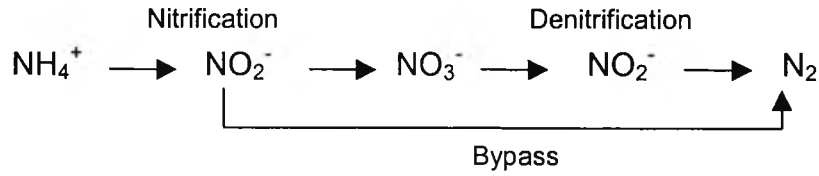
Source	DNR (gNO_3-N/m^2 d)	Reference
Fixed film column	0.05	Balderston & Sieburth(1976)
	0.08	Sauthier et al (1995)
Ponds	0.038	Gross et al (2000)
Fluidized bed column	**0.38**	**van Rijn et al. (1990; 1995)**
Airlift bioreactor	**0.03-0.05**	**This work**

Generally speaking, the performance of the PBABR in treating wastewater containing inorganic nitrogen compounds was comparable with other treatment systems, although it seems, at times, that the efficiency of nitrification/denitrification was not sufficiently high. On the other hand, it should not be overlooked that the PBABR offered both nitrification and denitrification in one step where both of the reactions took place at a comparable rate with other single treatment units. In other words, a complete nitrogen compound removal could be accomplished with one airlift bioreactor setup whilst most systems required a sequence of two reactors.

In addition, PBABR was shown to work well for a long period of time and it was not until the period of overgrown culture in the riser column that the system needed attention. The PBABR is also highly attractive due to the low energy and operating costs associated with the operation of the system. This analysis is not shown in this thesis.

However, it was found that nitrification and denitrification rates obtained from the PBABR were still unbalanced, i.e. the initial nitrification rate was far greater than the denitrification rate whilst the opposite was found when the experiment was left operating for a long time. This reason of unequal rates is described further below.

In this study, the exact mechanism how inorganic nitrogen was removed was still unknown as no thorough microbiological analysis was performed and only inorganic nitrogen (ammonium-nitrogen, nitrite-nitrogen and nitrate-nitrogen) were measured. However, it was assumed that proper nitrification and denitrification (Eqs. 2.1, 2.2 and 2.6) took place. In the actual situation, nevertheless the bypass process of nitrogen removal could potentially occur in the PBABR system where nitrite-nitrogen was converted directly to nitrogen gas, i.e.



In this process, ammonium-nitrogen was oxidized to nitrite-nitrogen by nitrifying bacteria and then it directly was reduced to nitrogen gas by denitrifying bacteria. This assumption is presented because there were times where nitrate-nitrogen was not found in PBABR during simultaneous nitrification denitrification operation.

Alternatively, nitrogen might have been assimilated by some microorganisms in the system. It was possible that, with the presence of methanol as a carbon source, some heterotrophic bacteria could grow where nitrogen uptake was occurred as one of the essential substrates. As a rough estimation, if the structure formula of bacteria was taken as $CH_{1.4}O_{0.4}N_{0.2}$, then the nitrogen required for cell syntheses was as much as 2.94 gN per 1 g of dry cell (Dincer, 2000). Some further analysis of the nitrogen uptake behavior still needs to be performed.

During the initial period of the experiment (0-80 hours), the inorganic nitrogen was found to increase. This could be due to the presence of shrimp culture medium that was still present in the sea culture. Shrimp culture medium was used as an inoculating solution and this culture medium contained high level of nitrogen. This culture medium was believed to be consumed by the microorganism where some of the nitrogen-compounds were dissimilated from this uptake mechanism.

4.2 Nitrification process

This section explained the nitrification obtained in the riser of the PBABR. The effect of various parameters of nitrification rates are presented in Figures 4.2.1 - 4.2.4 where details of these experiments are given below. In these figures, the x-axis shows the time of removal inorganic nitrogen and the y-axis shows the inorganic removal rate. It is worthwhile here to note that although the x-axis always started at zero, the actual time might not necessary be so. The data for these illustrations were taken from the various periods during the experiment, and as the data was re-plotted in Figures 4.2.1-4.2.4, the x-axis was always reset at zero to facilitate the data comparison.

4.2.1 Effect of alkalinity

Figure 4.2.1 illustrates the result of alkalinity on nitrification. The alkalinity as measured in mg $CaCO_3$/L was adjusted at approximately 27.5, 47.5, and over 100 mg$CaCO_3$/L. It was observed that the lack of alkalinity (below 100 mg$CaCO_3$/L, results at 1250-1376 hours) led to low ammonia removal rates (0.16-0.20 mg NH_4-N/m^2-d) compared to normal rates obtained from this work (approximated 0.43 mg NH_4-N/m^2-d). This is because alkalinity ($CaCO_3$) is essential as a carbon source for cell synthesis of nitrifying bacteria. Equation 2.4 shows the relation between ammonium and inorganic carbon as substrate for cell synthesis. Insufficient inorganic carbon resulted in no ammonium-nitrogen uptake. In addition, not only did inorganic carbon provide carbon source for cell synthesis and growth of nitrifiers, but it also did neutralize the hydrogen ions produced, which otherwise could cause a gradual decrease of pH during nitrification (Wallace and Nicholus, 1969; Sherrard, 1979; Koller and Avtalion).

Literature reported that nitrite-nitrogen could accumulate in the system at alkalinity below 101 mg$CaCO_3$/L which rendered the nitrite oxidation step a rate limiting step (Sakairi et al., 1996). However, results from this experiment indicated no nitrite accumulation at the period of low alkalinity (see Fig. 4.1.3 at 1118-1376 hours). Interestingly, nitrite accumulation only occurred at the period where there was nitrate accumulation (see Figs. 4.1.3 and 4.1.4). This means that nitrite was

rapidly consumed via denitrification process and the influence of alkalinity on the level of nitrite could not be seen in the system where both nitrification and denitrification took place simultaneously. The table below summarizes the nitrification rate (NR) obtained from experiments with various levels of alkalinity.

Alkalinity ($mgCaCO_3$)	NR (g NH_4-N/m^2 d)	Time period (h)
>100	0.870	144-198
48.44	0.192	1118-1207
27.5	0.157	1250-1376

4.2.2 Effect of pH

Table below shows the effect of pH on nitrification rate. Low nitrification rates were observed at pH below 7 (0-80 hours). This is because the nitrifying bacteria were very sensitive to pH and the optimum pH range was approximately 7.0-8.0 (Grady and Lim, 1980), where growth and activity of nitrifying bacteria decreased dramatically at a condition below neutrality (pH 7) (Hagopain and Reily, 1998).

pH	NR(g NH_4-N/m^2 d)	Time period (h)
below 7	0.216	0-80
over 7	0.870	144-198

4.2.3 Effect of dissolved oxygen

Figure 4.2.2 reveals that the dissolved oxygen in the riser section affected the nitrification rate. It was observed that the high dissolved oxygen (DO) concentration (DO > 4 mg/L during 144-198 hours) gave a higher nitrification rate (0.40 mg NH_4-N/L-h) compared to the rate at low dissolved oxygen level (DO < 4 mg/L). This is not an unexpected result as oxygen is well known as an essential requirement for the oxidation of ammonia by nitrifying bacteria (see Eqs. 2.1-2.4) (Oslislo et al., 1985; Compos et al., 1999).

At a DO level of 3.99-4.41 mg/L (during 144-198 hours), the maximum nitrification rate was obtained, but at the same time, nitrate accumulation was found which indicated that denitrification rate was significantly lower than nitrification rate. Denitrification was only found to take place at satisfactory rate (no nitrate accumulation) at the DO level of less than 2.66-3.15 mg/L (see Fig. 4.1.4 at 953-1113 hours). However, the nitrification rate at this time period was not as high as that obtained at higher DO levels (see Tables 4.2-4.3). This led to a conclusion that if the dissolved oxygen in the riser was too high, the oxygen uptake rate by nitrifying bacteria in the column was not enough to bring the oxygen down to the level suitable for denitrifying bacteria (0-2 mg/L). Therefore it is important to maintain a moderate DO level in the riser to ensure that both nitrification and denitrification could take place simultaneously.

DO (ppm)	NR(g NH_4-N /m^2 d)	Time period (h)
3.99-4.41	0.870	144-198
3.08-3.71	0.451	463-501
2.66-3.15	0.061	953-1113

4.2.4 Effect of Methanol

Figure 4.2.3 shows that the ammonium-nitrogen removal was affected by the addition of methanol. The result illustrated that the nitrification rate dramatically decreased with increasing methanol concentration. For example, nitrification at 541-619 hours where a high dose of methanol was added to the system was 0.217 g NH_4-N/m^2-h compared to the rate of 0.572 g NH_4-N/m^2-h at a low CH_3OH level of 0.16 % methanol (324-354 hours). Similar results were reported in literature where the ammonia removal rates were low at high organic carbon concentration. This is because high organic carbon and DO conditions were suitable for the growth of other heterotrophic microorganisms which could potentially compete with the nitrifiers in the uptake of oxygen. Besides, these heterotrophic microorganisms might also grow on the nitrifying biofilms leading to a decrease in nitrification rate (Bovendeur et al, 1990; Metcalf and Eddy, 1991; van Benthum et al, 1997; Matsuda et al., 1988; Satoh et al., 2000). This leads to a

conclusion that the nitrification process was strongly inhibited when organic carbon was present.

In this experiment, however, the addition of methanol was necessary for the growth of denitrifying bacteria. The level of methanol added into the system was adjusted to give the best results regarding denitrification rate. However, it was realized that this methanol would adversely affect the nitrification rate. Literature shows that the total ammonia nitrogen removal rate could be reduced by almost 70% when the carbon to nitrogen ratio (C/N ratio) was 1.0-2.0 compared with a pure nitrification process (C/N=0) (Zhu and Chen, 2001). Table below shows the effect of methanol on nitrification rate.

% Methanol (%v/v)	NR(g NH_4-N /m^2 d)	Time period (h)
0	0.870	144-198
0.16 (C/N = 25)	0.572	324-354
1.0 (C/N = 62)	0.217	541-619

4.2.5 Effect of biofilm ages

Figure 4.2.4 shows the results of ages of nitrifying biofilms on nitrification. Younger biofilm with an age at 144-204 hours gave a higher nitrification rate (0.572 gNH_4-N/m^2-d) than older biofilm with an age of 625-763 hours (0.137 gNH_4-N/m^2-d). Visual observation during experiment found that the nitrifying biofilms grew steadily with time and after some period of time, the biofilm was overgrown and made it difficult for the air bubble to transport through the plastic packing. This effectively means a higher diffusional mass transfer resistance for oxygen on the surface of the packing. In other words, oxygen needed to diffuse through a thicker biofilm layer to reach the nitrifying bacteria resided in the deep layer of the biofilm. Hence, only the outer layer bacteria were exposed to high DO condition, whereas the inner layer bacteria were subject to the low DO condition. This consequently reduced the nitrification performance in nitrification section. This oxygen limitation due to biofilm thickness is similar to the reported of Zhang et al. (1995); Schramm et al. (1996). The report of van Benthum et al. (1996)

stated that, in using the microelectrode studies on tricking filter biofilms, no nitrification could take place under 100-150 μm layer. However, this result was not verified for this work. The table below shows the nitrification rate at various biofilm ages.

Ages of biofilm (hours)	NR(g NH_4-N /m^2 d)	Time period (h)
144	0.870	144-198
324	0.572	324-354
541	0.217	426-456
625	0.137	625-763

4.3 Denitrification process

Figures 4.3.1-4.3.4 reveal the influence of various parameters on denitrification. Results are extracted from the reactor performance at various time periods.

4.3.1 Effect of dissolved oxygen

Figure 4.3.1 illustrates that the nitrate removal depended on dissolved oxygen level in the downcomer of the PBABR. Denitrification was found to occur at a rather high DO of about 2.5-3.0 mg/L (experiment at 463-501 hours) but with a low rate (0.013 gNO_3-N/m^2-d) compared to the rate at low dissolved oxygen ranges (0.03-0.08 gNO_3-N/m^2-d). It indicated that the nitrate removal rate decreased with increasing oxygen concentration. This result is similar to the observation of Hagedorn-Olsen et al (1994) who reported that the maximum nitrate removal rate decreased with increasing oxygen concentration. This result is, in fact, not surprising as it is well known that denitrifiers are facultative aerobes with the ability to use both oxygen and nitrate as electron acceptor in their metabolic processes. Denitrifying bacteria prefers oxygen as electron acceptor because they obtain high energy per mole of oxygen consumed (US. EPA, 1975). Hence, in the presence of oxygen, denitrifying bacteria would not consume nitrate as an electron acceptor. Painter (1977) reported that at sufficiently low level of

dissolved oxygen (0.2-1.5 mgO_2/L), denitrifying bacteria was found to switch from using oxygen to nitrate as electron acceptor. In this work, the results illustrated that denitrification could occur at a low dissolved oxygen range of 0.39-2.78 mg/L with the rate as summarized below.

DO (ppm)	TNR(g N/m^2 d)	Time period (h)
0-1.5	0.049	955-1125
1.5-2.0	0.034	1555-1598
2.5-3.0	0.013	463-501

It is noted that the oxidation-reduction potential (ORP) range in the downcomer of the PBABR was found to be in between -10 to +150 mV. This range of ORP was reported to be the condition where denitrification could occur without generating toxic byproducts such as H_2S (Balderson and Sieburth, 1976). However, the range of ORP was not definite and investigators reported different levels of ORP for denitrification. For instance, Lee (2000) suggested ORP range between -200 to -400 mV for a proper control of denitrification process.

4.3.2 Denitrification with addition of methanol

Figure 4.3.2 shows the results of the methanol addition on denitification rate. During 1471-1544 hours, 200 mL of 95% methanol was added to the system, and the denitrification rate was found to be 0.028 g NO_3-N/m^2-d. This was lower than the rate obtained when more methanol was added to the system (i.e. 0.030 g NO_3-N/m^2-d with 600 mL of 95% methanol during 541-619 hours). It should be noted here that the ratio between carbon and nitrogen (C/N ratio) suitable for denitrification reported in literature was about 1-2 (Balderson and Sieburth, 1976; Lee, 2000). However, the results from this work suggested that this value be as high as 20-40 (with 200mL of 95% methanol added into the system) where lower C/N ratio did not seem to promote denitrification. The reason for this is still unknown.

The effect of methanol addition was complicated. Experimental results illustrated that if the system was started with no methanol, denitrification could hardly take place unless methanol was added into the system at adequate dose. However, preliminary experiment in the PBABR with small size downcomer indicated that denitrification could not take place at all, despite the addition of methanol. This was because it was not possible to reduce the DO level in the downcomer of this small downcomer PBABR while remaining a high DO level in the riser. It is therefore believed that the addition of methanol was closely related with the decrease of dissolved oxygen in the downcomer (also can be extracted from Figure 4.1.7). Methanol added in PBABR was separated into two parts. One was used as organic carbon source by heterotrophic bacteria which also consumed dissolved oxygen. This process decreased dissolved oxygen down to the level suitable for denitrification reaction and then the denitrifying bacteria could function. Hence, it is important that the system was properly designed to ensure a smooth operation of both nitrification and denitrification reactions.

Table below shows the effect of methanol addition in the system. The suitable level of methanol needed for nitrate removal was found to be higher than 200mL (or C/N ratio of 20-40). Insufficient addition of methanol (0-0.17%v/v) led to the nitrate accumulation in system.

%Methanol (v/v)	TNR(g N/m^2 d)	Time period (h)
0	0	426-456
0.17	0	1413-1471
0.3	0.028	1471-1544
1.0	0.030	541-619

It is interesting to mention here that when the system was left operating for a long period of time (e.g. at period during 775-1125 hours), nitrate-removal could occur without an addition of carbon source. In other words, there was no need to add methanol at all and denitrification could still take place perfectly. It is believed that there was an overgrown bacteria (can be either denitrifying or other heterotrophic bacteria) in the system and some would undergo endogenous

decay mechanism. These dead microorganisms might be the source of carbon for other living denitrifier in the system. Hence, the system could be operated without the addition of external carbon source like methanol.

4.3.3 Effect of nitrogen gas purging on denitrification

Figure 4.3.3 reveals the result of purging of N_2 gas on denitrification process in this work. At initial period (150-354 hours), the purging of N_2 gas aided the venting of the dissolved oxygen in system. The results observed that the 100 % of nitrogen removal achieved during this N_2 purging period (150-190 hours) where only 0.25% by vol of methanol was needed for the denitrification (in non-purging N_2 period or during 463-619 hours, more methanol, 1% by vol, was required). This result was attributed to the fact that nitrogen gas purging resulted in a decrease in the dissolved oxygen in the downcomer of the PBABR, a condition favorable for denitrification.

N_2	%Methanol (v/v)	TNR(g N /m^2 d)	Time period (h)
Y	0.25	0.045	150-190
N	1	0.030	541-619

4.3.4 Effect of biofilm ages

As stated earlier that it was rather cumbersome to measure the denitrification rate in this system as nitrate was one of the intermediates in the nitrogen removal process. However, the denitrification rate of denitrifying bacteria was observed to be quite low compared with that of nitrifying bacteria and no overgrown biofilm was observed in the downcomer of the PBABR. Figure 4.3.4 displays the total nitrogen removal rate (TNR) with time at various biofilm ages. It was found that the TNR was not significantly affected by the biofilm age during the first 1471 hours. At the 1677 hours, the TNR was found to be much greater (with thicker bacteria). However, Figure 4.2.4 illustrated that the nitrification rate was slower with a thicker biofilm which means ammonia was removed at a slower rate. These two findings led to a conclusion that the denitrification rate must have taken

place at a much faster rate as the biofilm became older. It might be because the bacteria already acclimatized themselves to the condition of the reactor which accelerated the nitrate removal rate. And also it might be possible that there existed a larger quantity of bacteria in the old biofilm which led to an increase in the denitrification rate.

It should also be included here that the thick biofilm in the riser helped reduce the dissolved oxygen in the downcomer section of the PBABR became quite low and this facilitated the growth of the denitrifying bacteria. The other possible scenario for the increasing rate of denitrification with biofilm ages or thickness rather was that there existed some other bacteria in the biofilm in the riser section which consumed nitrate-nitrogen as their essential substrate resulting in a reduction in nitrate nitrogen.

4.4 Concluding remarks

The PBABR was found to be suitable for the treatment of wastewater containing nitrogen compounds. The condition favorable for nitrification and denitrification in this PBABR was summarized as follows:

Operating condition	Nitrification	Denitrification
pH	7-8	7-8
Alkalinity ($mgCaCO_3$)	>100	-
DO [ppm]	3-4	0.3-2
ORP [mV]	-	-10 to 150
Initial C/N	-	20-40
% Methanol (v/v)	-	0.3-1

Table 4.1 shows the operating condition periods during experiment

Experimental period (h)	Condition	Run
0-80	NH_3-N 8.7 mg/L	0
144-204	NH_3-N 8.7 mg/L, methanol 0.25 % (v/v), N_2	0
324-405	NH_3-N 8.7 mg/L, methanol 0.16 % (v/v), N_2	1
426-456	NH_3-N 8.7 mg/L, methanol 0.16 % (v/v)	1
464-501	NH_3-N 8.7 mg/L, methanol 0.16 % (v/v)	1
541-619	NH_3-N 8.7 mg/L, methanol 1% (v/v)	1
625-763	NH_3-N 8.7 mg/L, methanol 1% (v/v)	1
775-930	NH_3-N 8.7 mg/L, NO_3-N = 5 mg/L (892 h)	1
953-1112	NH_3-N 8.7 mg/L, NO_3-N = 10 mg/L	1
1118-1207	NH_3-N 8.7 mg/L, NO_3-N = 10 mg/L	2
1213-1544	NH_3-N 8.7 mg/L, methanol 0.33% (v/v) (1471 h)	2
1550-1597	NH_3-N 8.7 mg/L, NO_3-N = 10 mg/L	2
1613-1671	NO_3-N = 10 mg/L	2
1677-1787	NH_3-N 8.7 mg/L, NO_3-N = 20 mg/L	2
1812-1812	NH_3-N 8.7 mg/L, NO_3-N = 20 mg/L, methanol 0.33% (v/v)	3

*Run = The number of run with new solution
*v/v = Volume of 95% methanol volume per volume of solution

Table 4.2 Summary of nitrification rate during experiment

Experiment hour	NH_4-N (mgN/L)		NR		pH	DO (mg/L)	Alkalinity ($mgCaCO_3$/L)	Methanol addition (mL)
	initial	final	mgN/L-h	gN/m^2-d				
0-80	8.959	1.624	0.098	0.216	6.32	7.296	>100	0
144-198	9.633	0.052	0.395	0.870	7.18	4.17	>100	200
324-354	5.305	0	0.260	0.572	7.17	3.08	>100	100
426-456	6.359	0.017	0.248	0.546	6.99	3.97	>100	100
463-501	7.731	0	0.205	0.451	6.73	3.51	>100	100
541-619	10.913	0	0.099	0.217	6.87	3.64	>100	600
625-763	7.963	0	0.062	0.137	7.21	3.3	>100	600
775-930	9.837	0	0.060	0.133	7.3	3.49	>100	0
953-1113	8.941	0	0.028	0.061	7.42	3.06	>100	0
1118-1207	12.743	6.341	0.087	0.192	6.96	3.62	48.44	0
1213-1244	9.938	1.996	0.094	0.207	7.45	4.29	47.5	0
1250-1376	11.157	0	0.071	0.157	7.39	4.71	27.5	0
1555-1670	8.539	0.199	0.097	0.214	7.45	3.85	>100	200
1677-1787	7.69	1.322	0.081	0.179	7.5	4.07	>100	0.00
1812-1878	8.154	1.282	0.058	0.128	6.82	4.2	>100	200

*N as NH_4-N

Table 4.3 Summary of denitrification rate during experiment

Experiment Hour	NH_4-N (mgN/L)		Rate		DO (mg/L)		ORP (mV)		Methanol addition	N_2
	initial	final	(mg N/L-h)	(gN/m^2-d)	L	R	L	R	(mL)	
150-190	4.72	0	0.413	0.045	2.65	2.04	134.02	135.12	200	Y
324-354	14.11	7.23	0.088	0.100	1.73	1.81	148.90	149.16	100	Y
463-501	10.01	14.39	0.118	0.013	2.50	2.78	155.25	154.83	600	N
541-619	15.55	5.04	0.270	0.030	1.81	1.78	145.94	145.59	600	N
955-1125	10.69	0.56	0.500	0.049	1.17	0.39	99.10	101.65	0	N
1471-1544	24.08	0.87	0.253	0.028	2.29	2.24	98.71	96.63	200	N
1555-1598	12.88	2.72	0.305	0.034	1.4	1.68	100.95	100.82	0	N
1613-1670	12.96	2.99	0.386	0.043	1.582	0.952	92.56	89.54	0	N
1677-1787	21.35	0	0.726	0.080	1.5925	0.8225	95.30	95.15	200	N

*N as NO_3-N

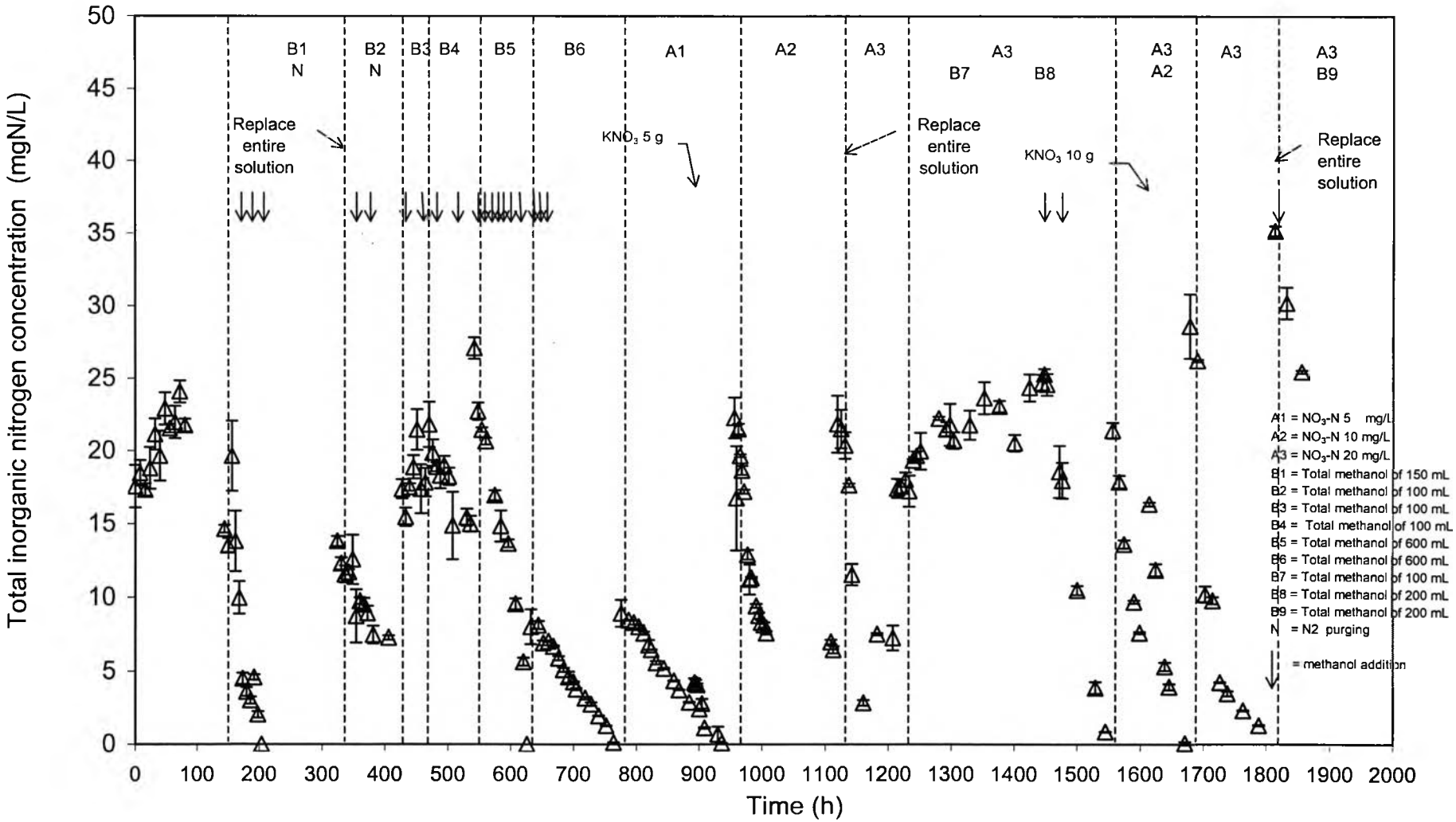


Figure 4.1.1 The total inorganic nitrogen compound profile during experiment

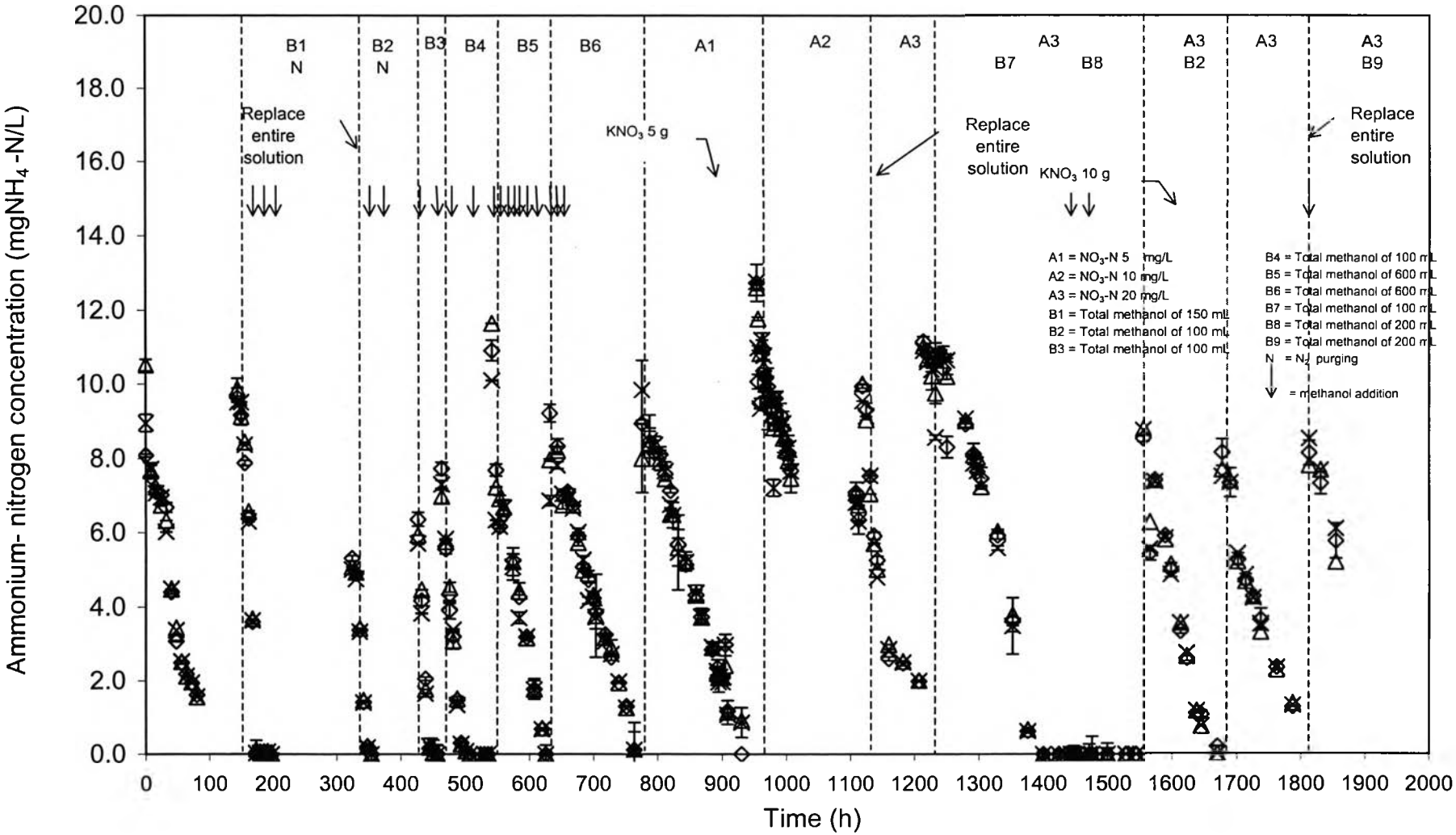


Figure 4.1.2 Ammonium-nitrogen concentration profile during experiment

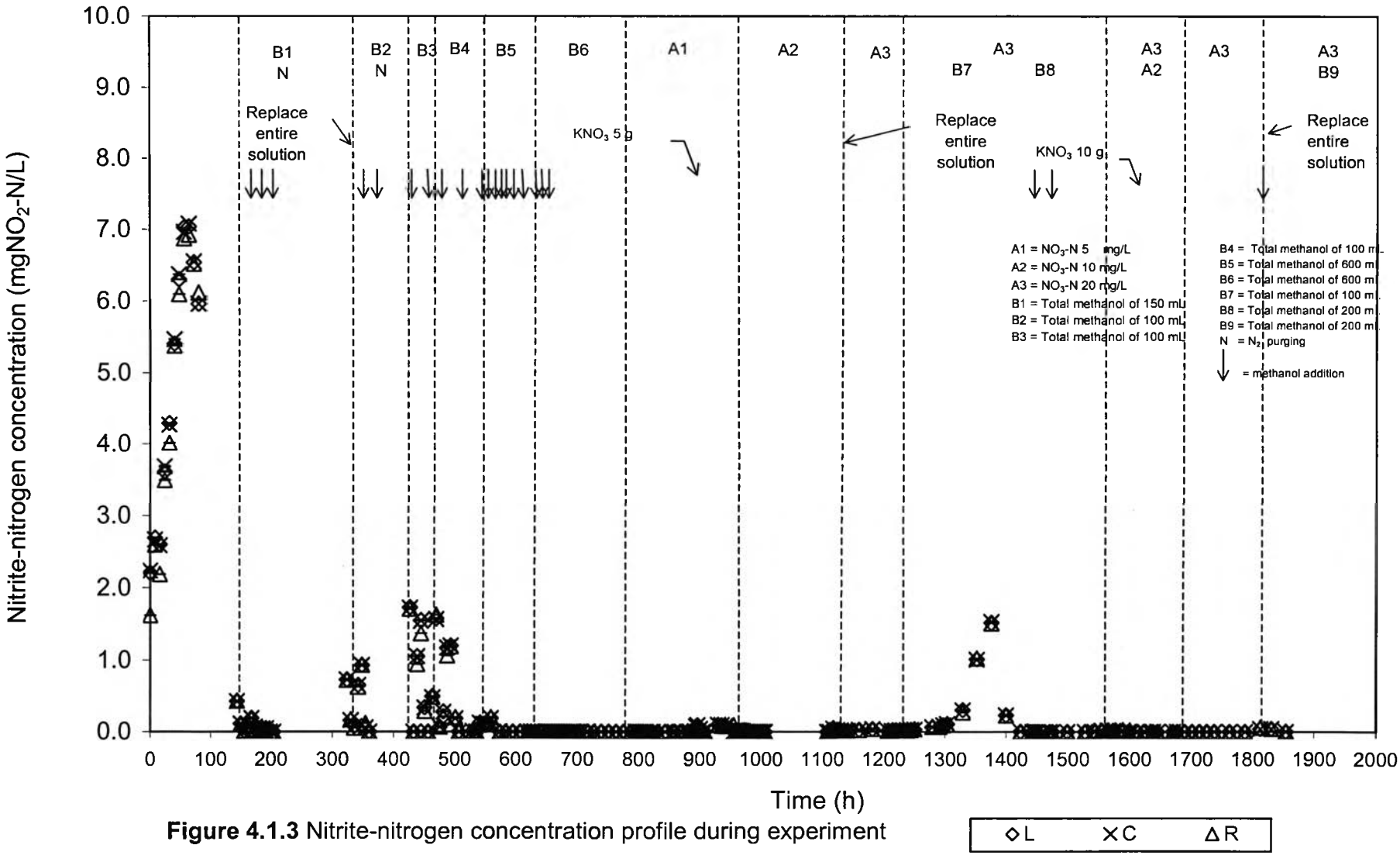


Figure 4.1.3 Nitrite-nitrogen concentration profile during experiment

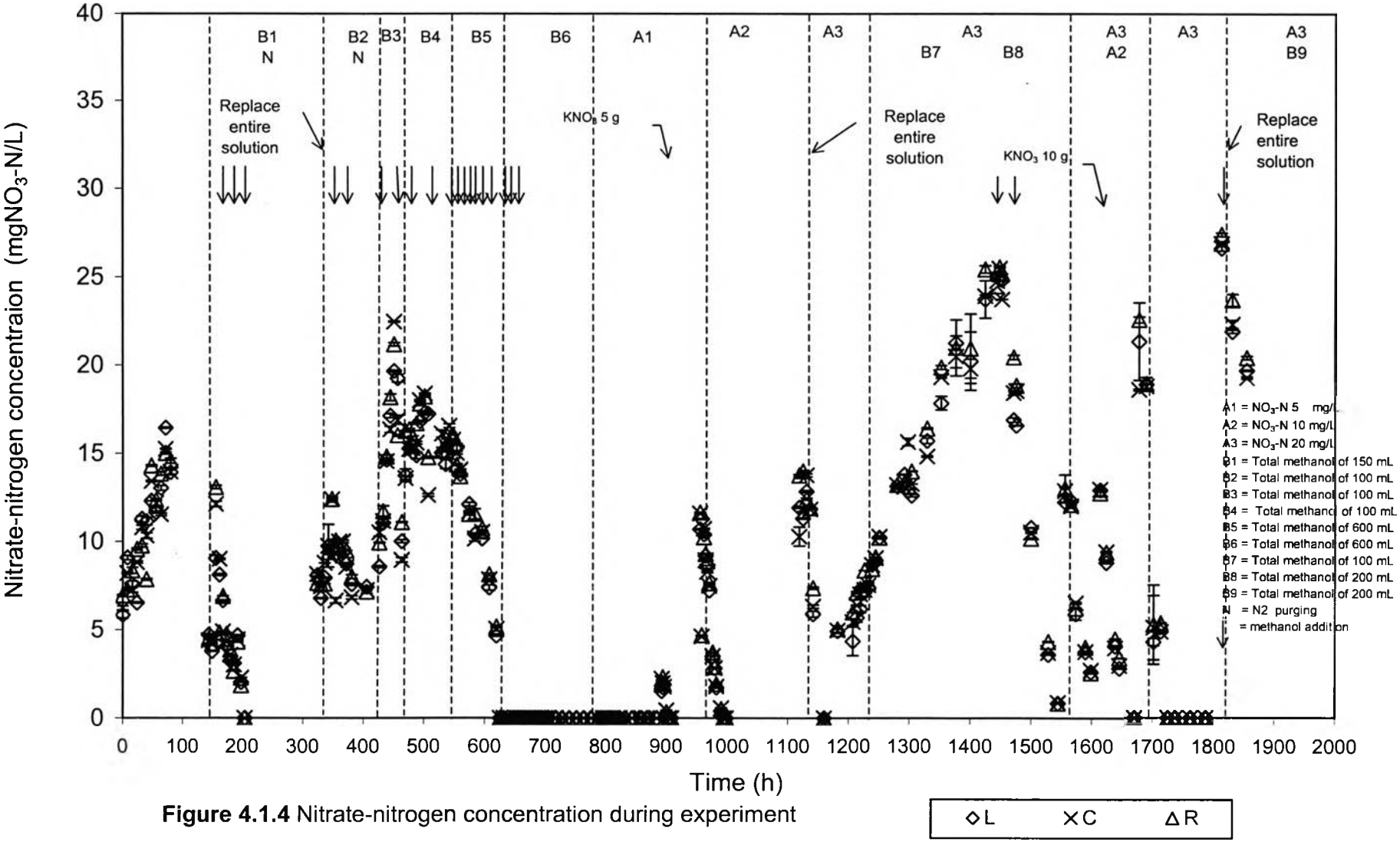


Figure 4.1.4 Nitrate-nitrogen concentration during experiment

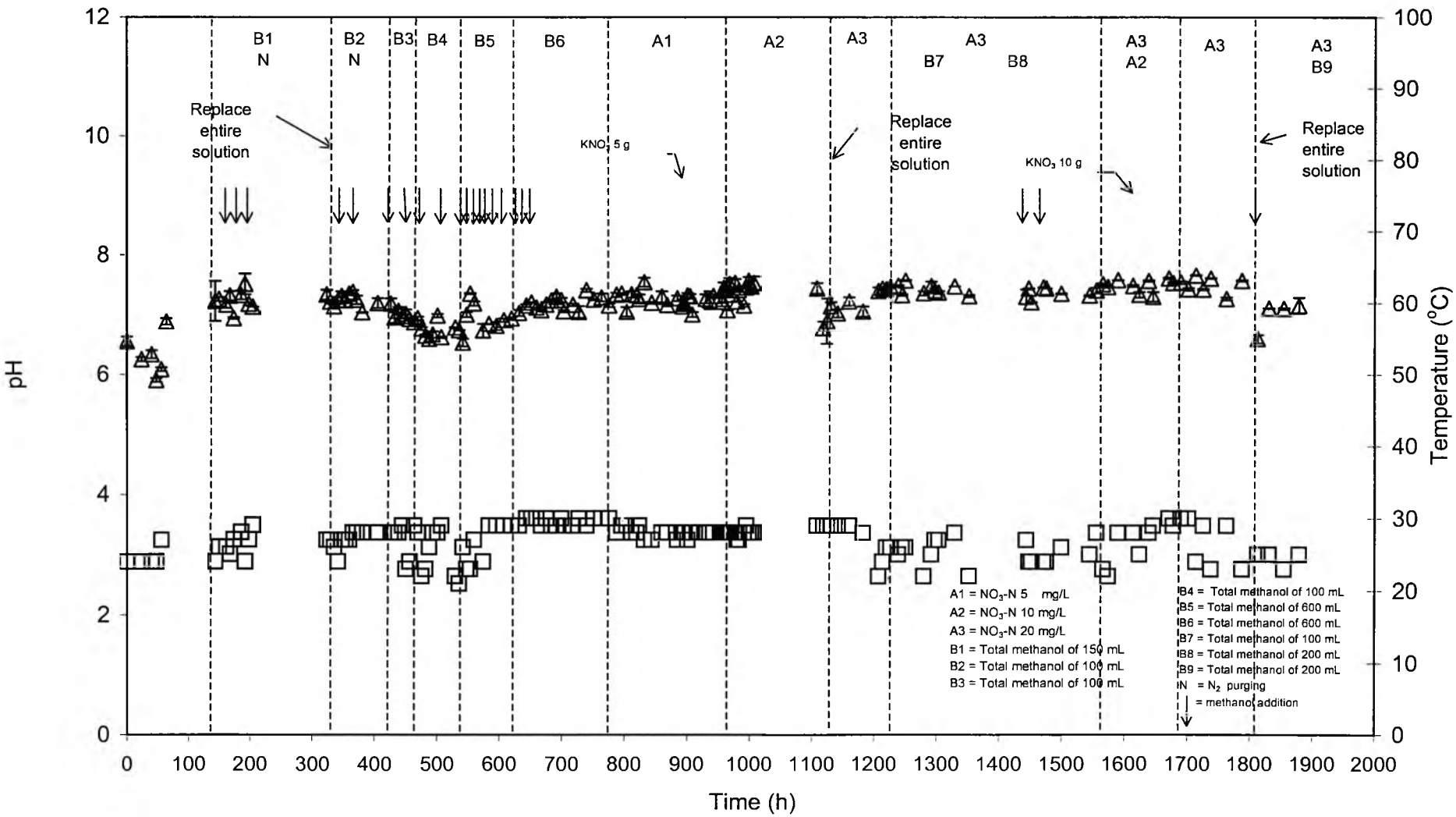


Figure 4.1.5 pH and Temperature of effluent during experiment

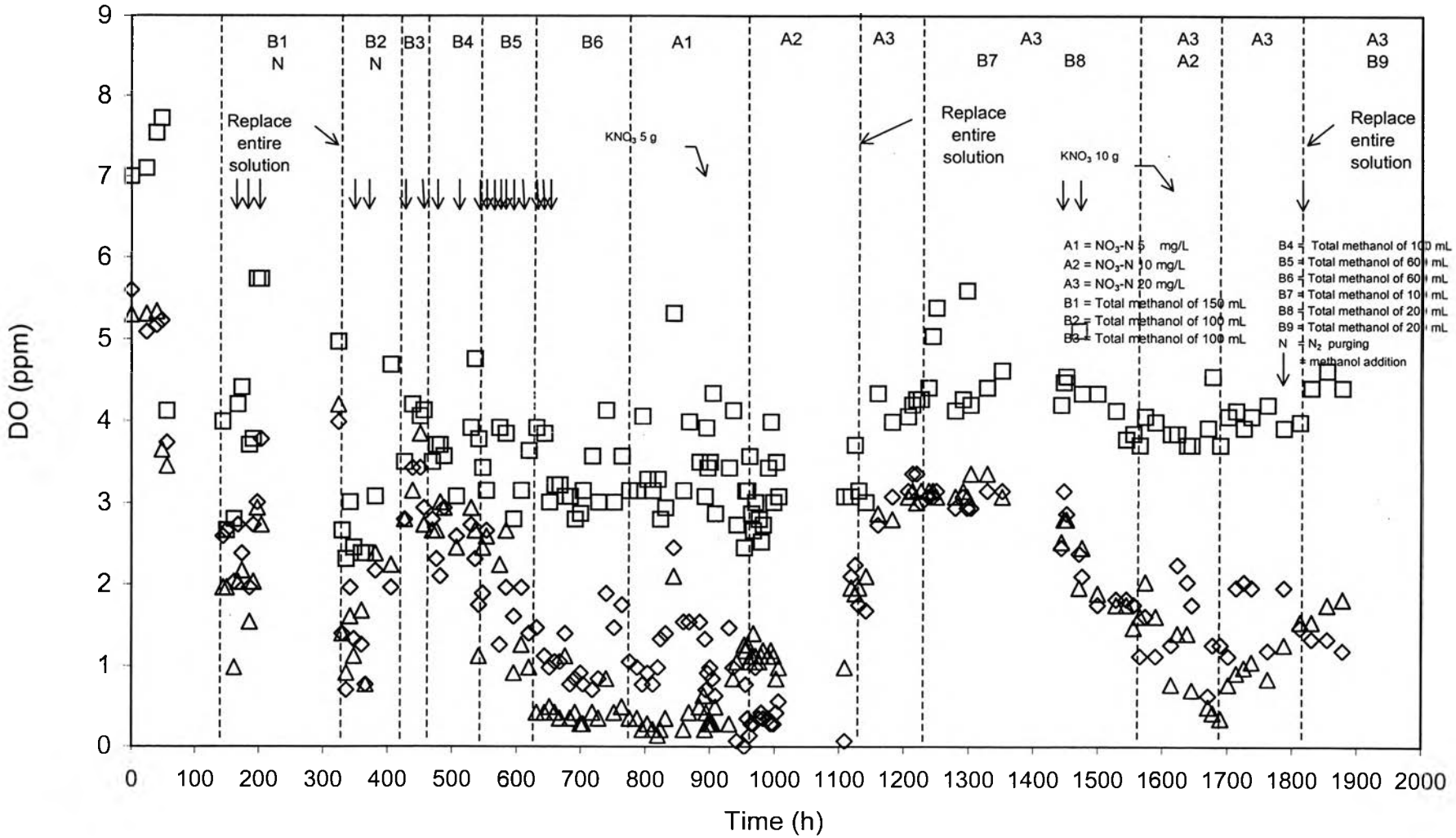


Figure 4.1.6 Dissolved oxygen of effluent during experiment

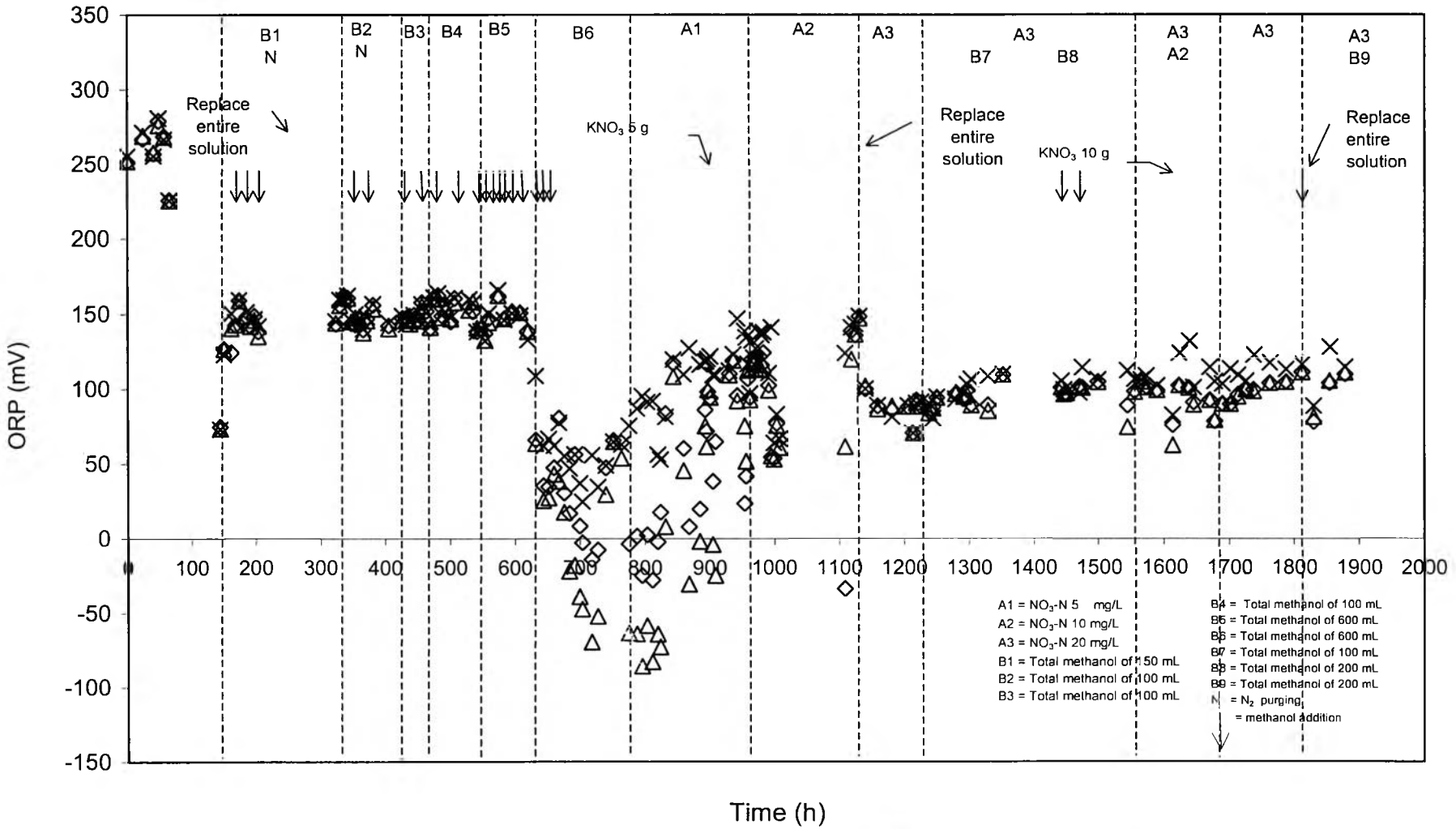


Figure 4.1. 7 Oxidation-reduction potential during experiment

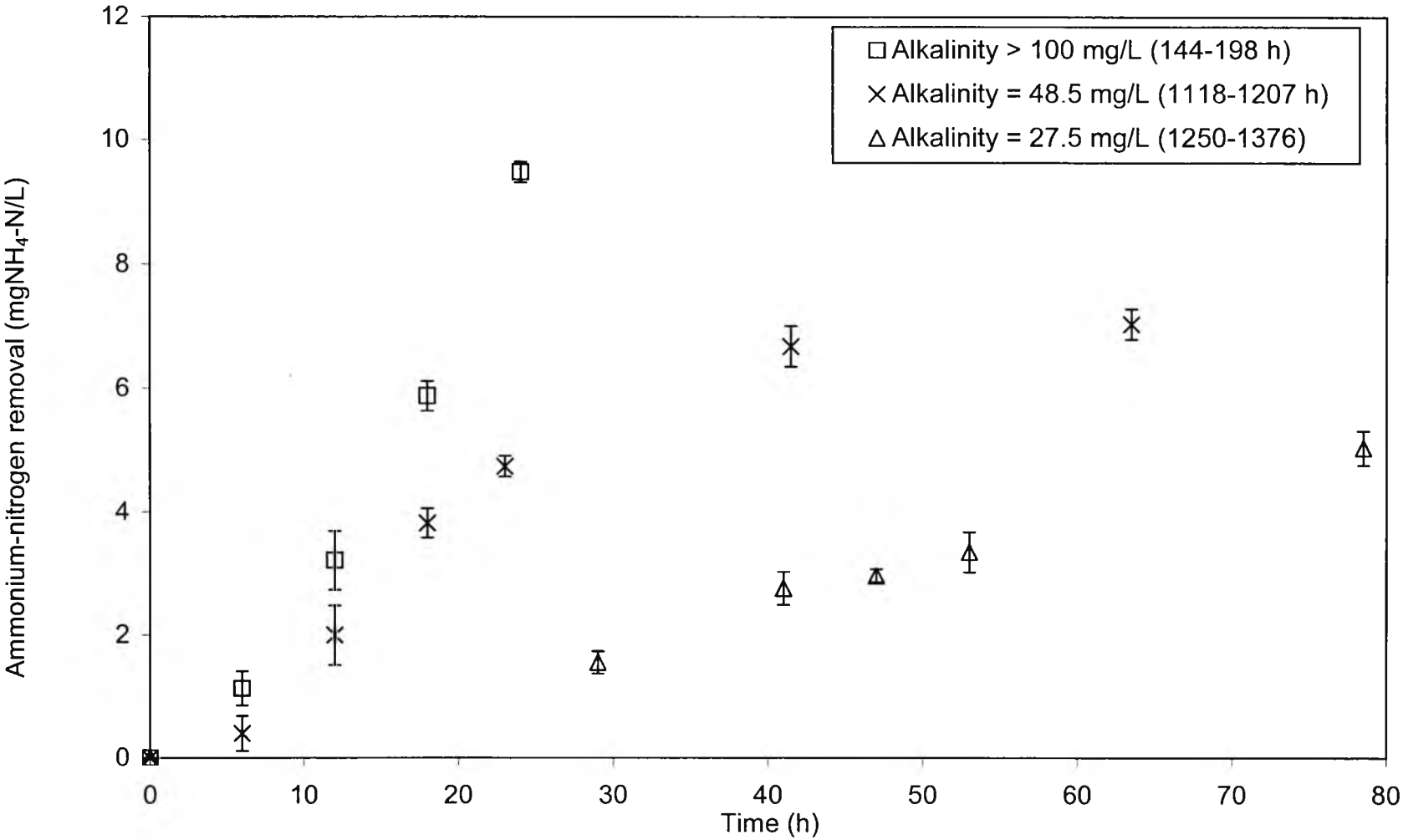


Figure 4.2.1 Ammonium-nitrogen removal at difference level of alkalinity (measure as $CaCO_3$)

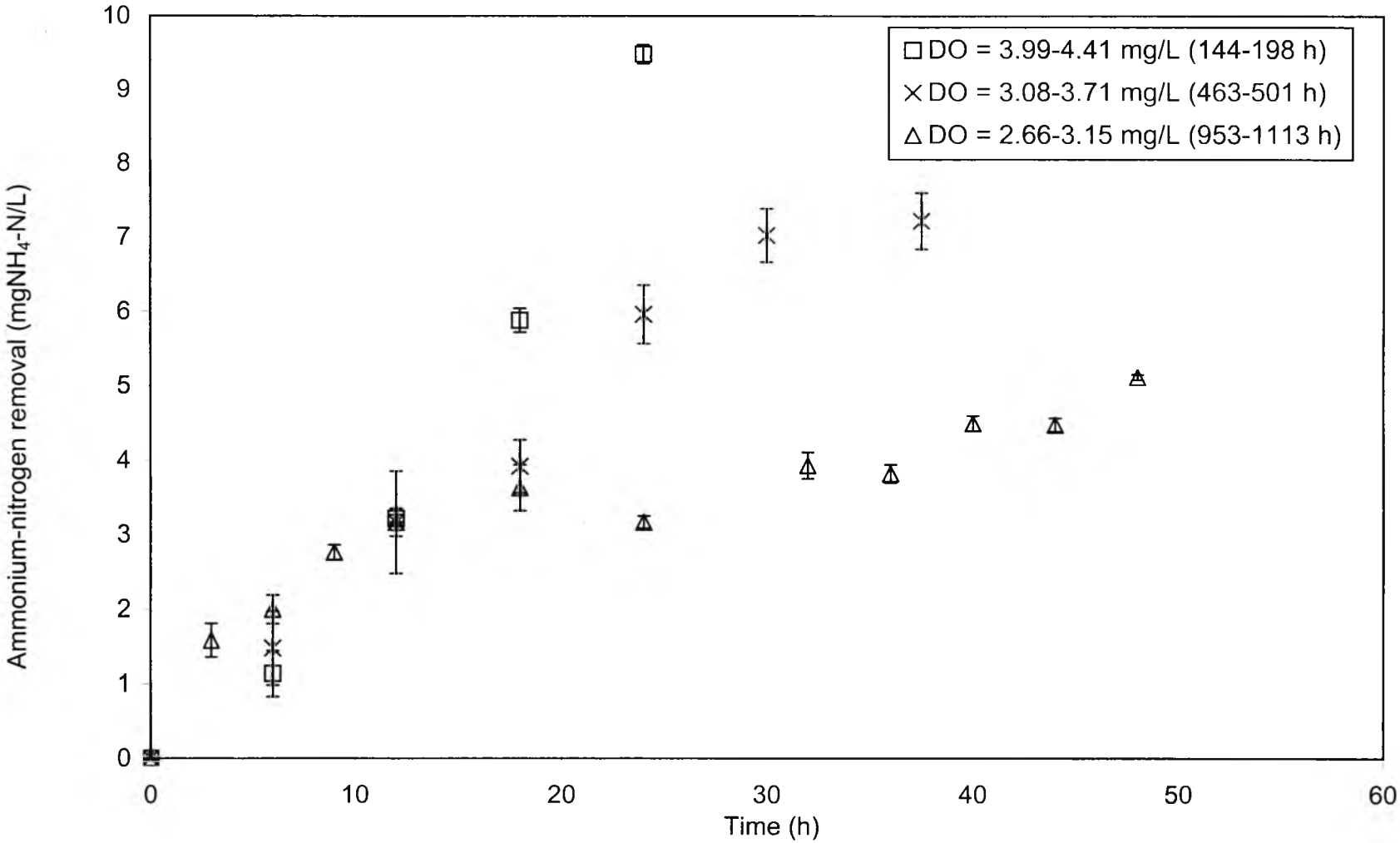


Figure 4.2.2 Ammonium-nitrogen removal at difference dissolved oxygen level in riser section

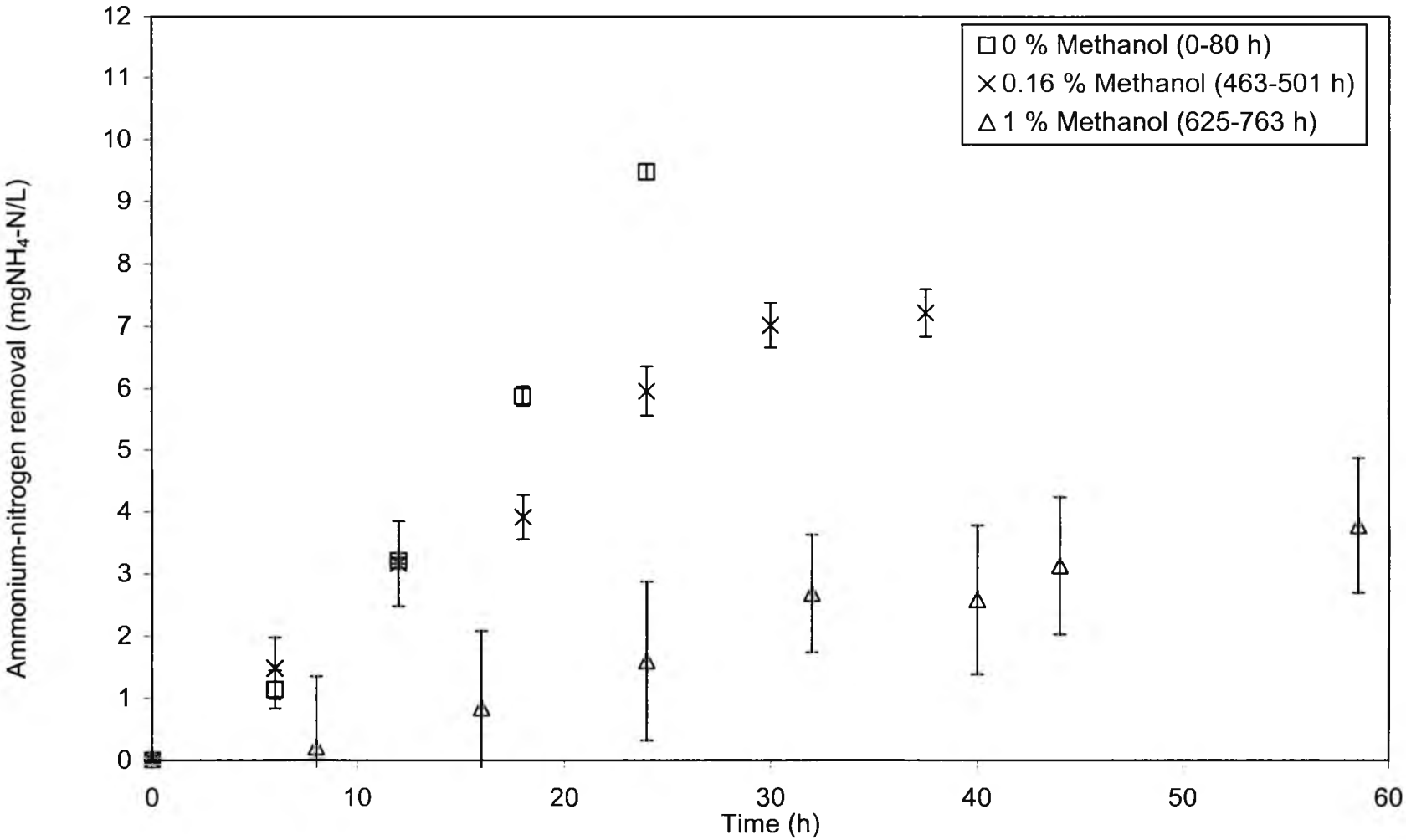


Figure 4.2.3 Ammonium-nitrogen removal at difference level of total methanol

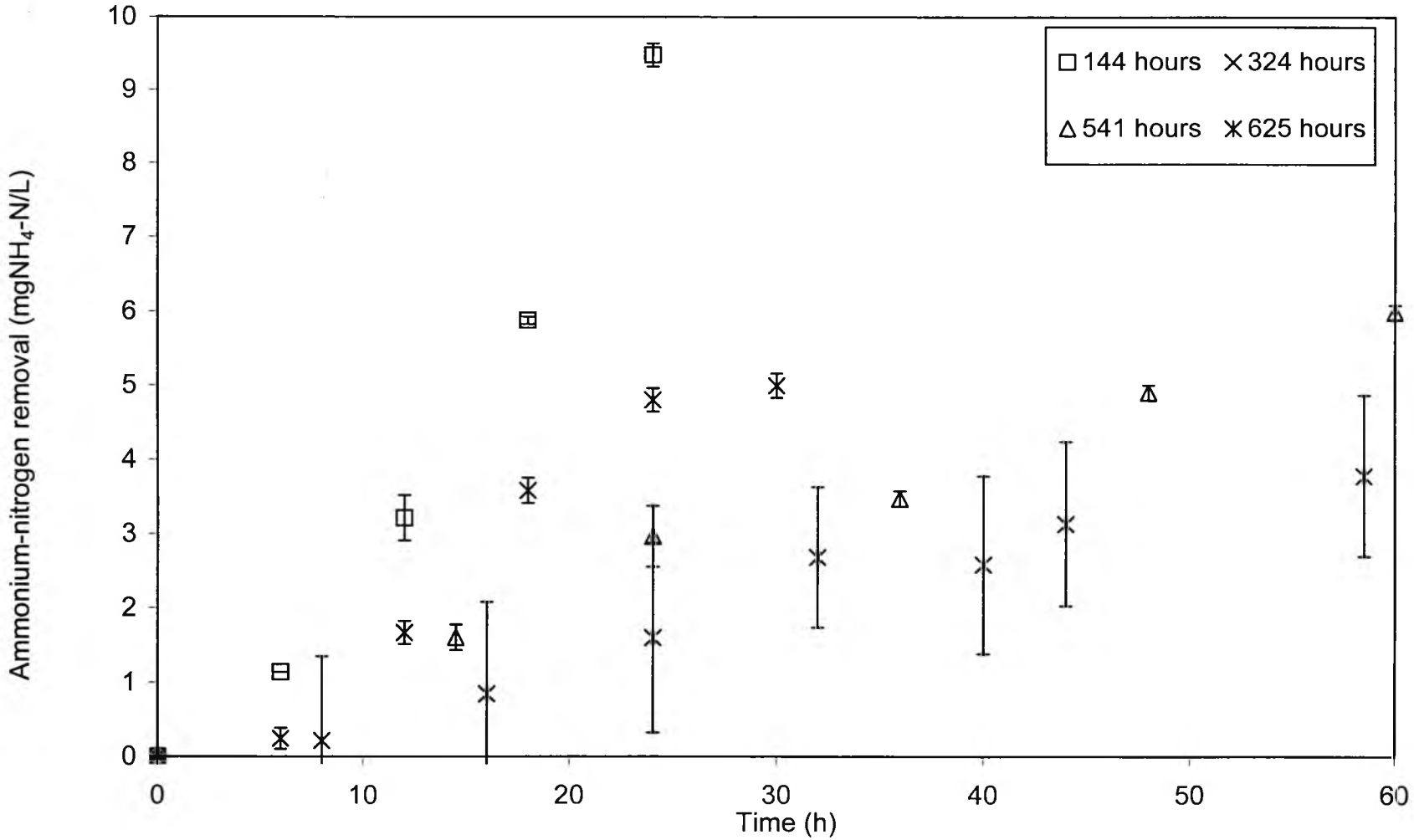


Figure 4.2.4 Ammonium-nitrogen removal at difference biofilm ages

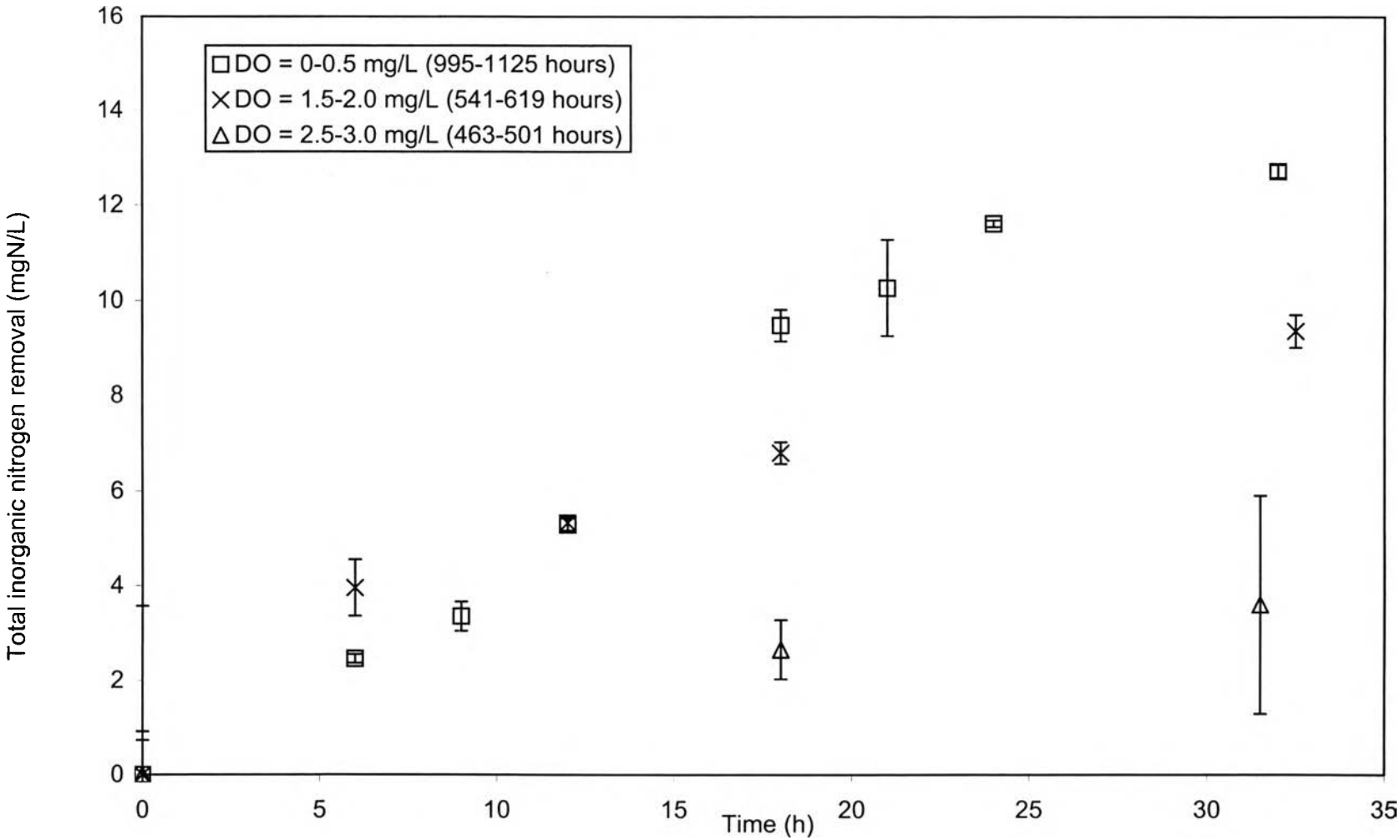


Figure 4.3.1 Total nitrogen removal at difference dissolved oxygen level in downcomer section

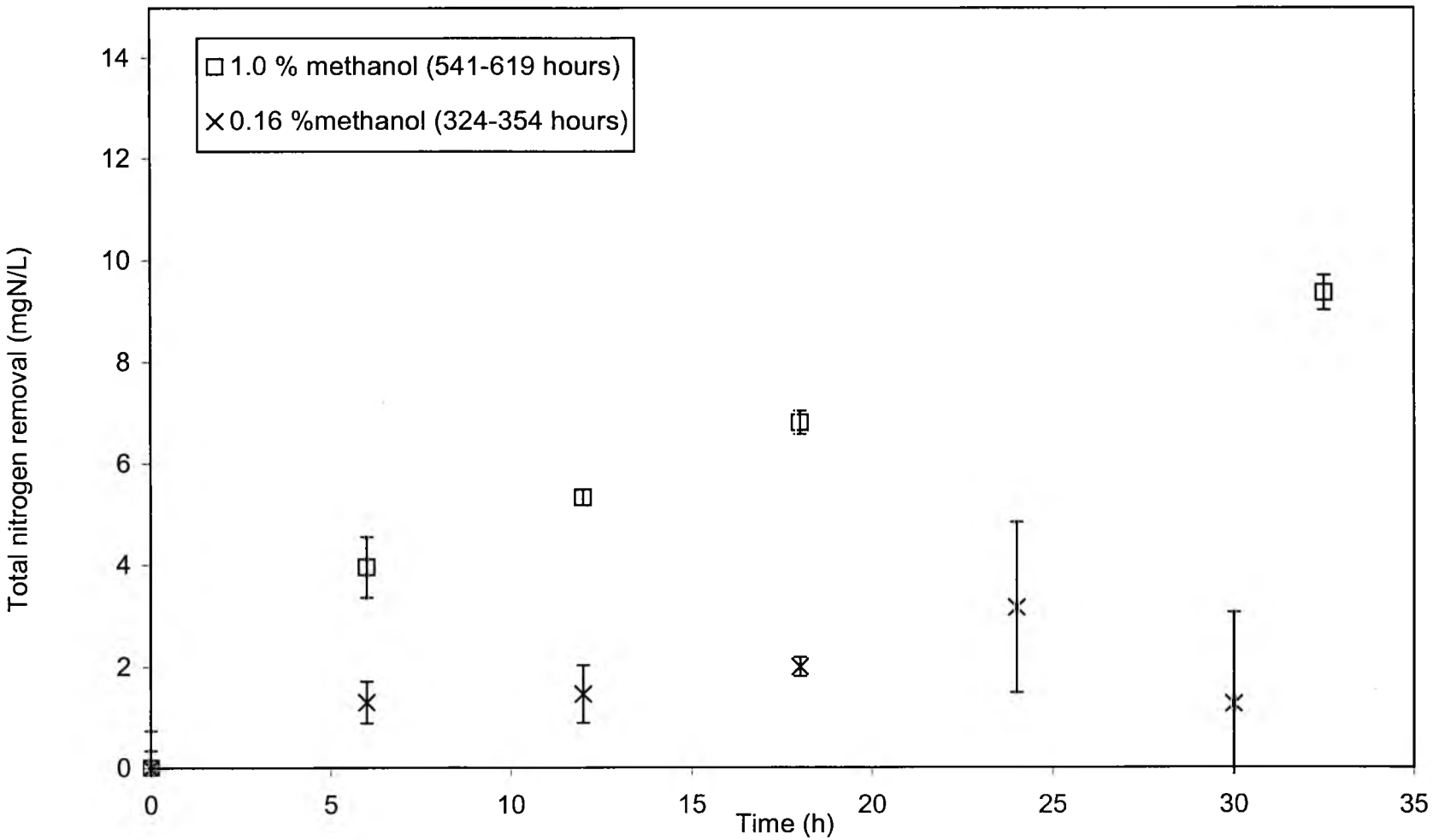


Figure 4.3.2 Total nitrogen removal at difference level of total methanol

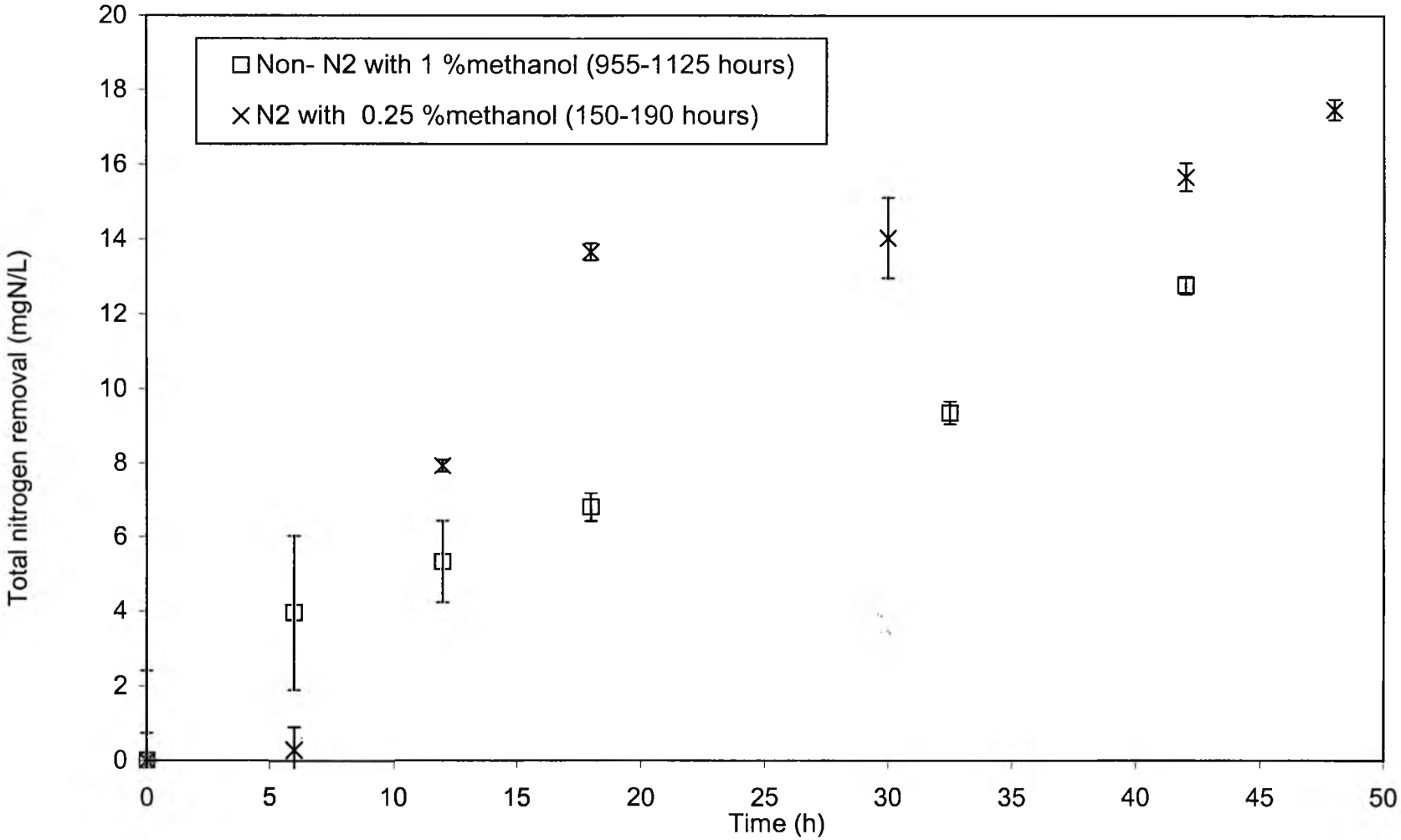


Figure 4.3.3 Total nitrogen removal with purging/non-purging nitrogen gas period

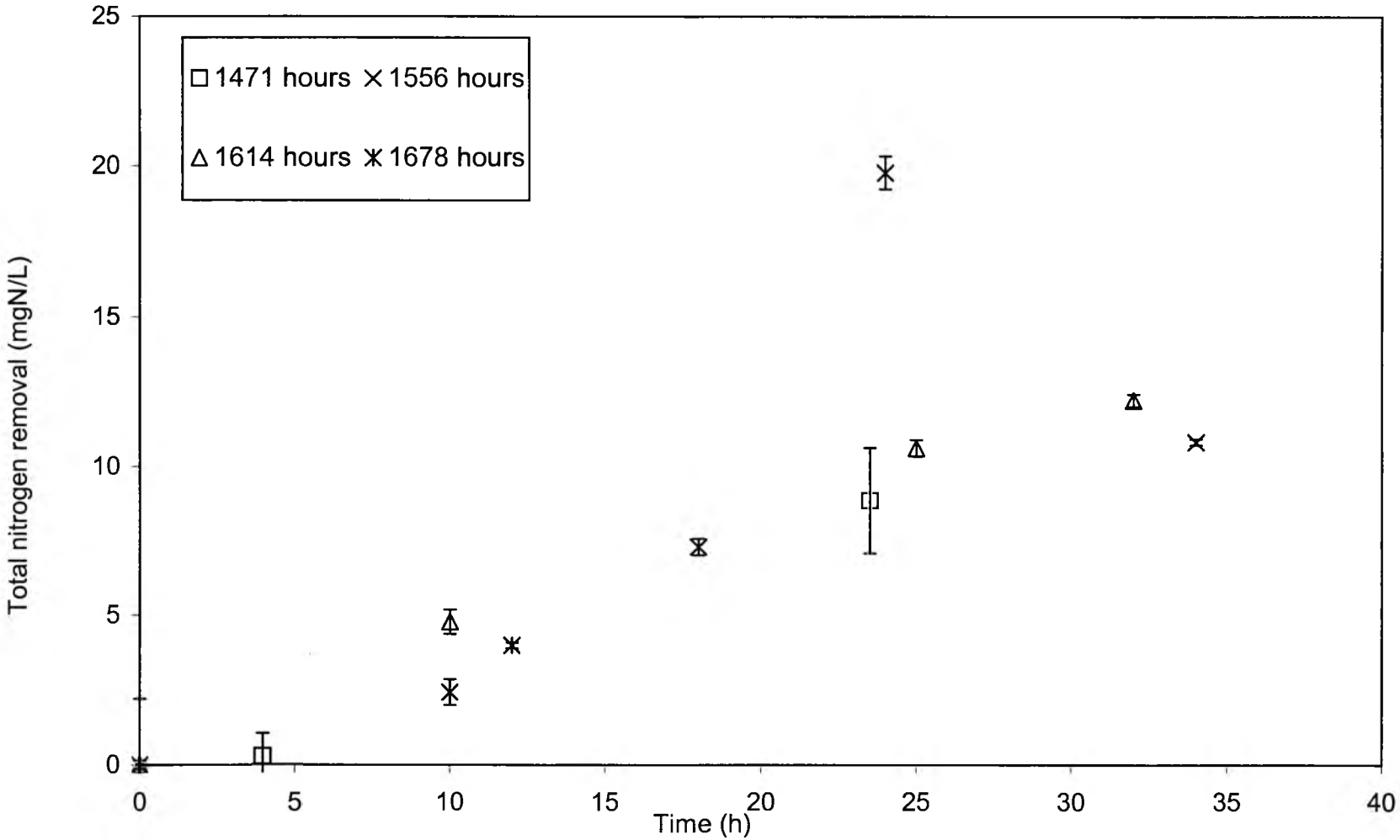


Figure 4.3.4 Total nitrogen removal at difference biofilm ages